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Thesis of a doctoral thesis

**OPTIMISATION OF INDOOR CULTIVATION
OF HEMP *CANNABIS SATIVA L.* TO INCREASE
FLOWER AND CANNABINOID YIELD**

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Abstrakt

Rostlinu konopí je možné pěstovat již desítky let, ačkoli je tato rostlina považována za návykovou látku v mnoha zemích na světě a to jak její technická tak léčebná varieta. Dříve běžně pěstované rostliny jsou klasifikovány jako technické konopí, tedy konopí s obsahem pod 1 % THC, nicméně v posledních letech je možné komerčně pěstovat i tzv. konopí pro léčebné použití, tedy konopí které může obsahovat i více než 20 % tohoto kanabinoidu.

V České republice je možné konopí pro léčebné použití pěstovat pouze ve zděných budovách s řízeným klimatem bez přístupu denního světla, to znamená, že pěstitelé musí rostliny pěstovat jinak než na polích a musí veškeré vstupy a parametry ovládat, což vyžaduje značnou míru porozumění daného pěstebního systému.

Jedněmi z nejvíce diskutovaných parametrů, které jsou přímo ovládány pěstiteli je světlo, jeho kvalita a kvantita a hnojiva. Hnojiva (jeho různé koncentrace) a světlo (jeho rozdílná intenzita) byly předmětem této práce.

V tomto experimentu byly rostliny konopí pěstovány v kontrolovaném prostředí, kde byly pěstovány celkem dvanáct týdnů. Tyto rostliny byly podrobeny dvěma režimům hnojení, R1 a R2.

Režim hnojení R1 byl aplikován pro celý vegetační cyklus (vegetační a kvetoucí fáze) s parametry N-NO_3^- 131.26 mg L⁻¹; N-NH_4^+ 6.23 mg L⁻¹; P_2O_5 30.85 mg L⁻¹; K_2O 112.46 mg L⁻¹; CaO 147.90 mg L⁻¹; MgO 45.72 mg L⁻¹; SO_4^{2-} 33.79 mg L⁻¹. Režim hnojení R2 byl rozdělen na vegetační fázi a fázi květu. Ve fázi vegetace (první čtyři týdny) bylo množství hnojiva aplikováno při N-NO_3^- 164.99 mg L⁻¹; N-NH_4^+ 5.28 mg L⁻¹; P_2O_5 65.87 mg L⁻¹; K_2O 228.27 mg L⁻¹; CaO 125.42 mg L⁻¹; MgO 78.93 mg L⁻¹; SO_4^{2-} 50.16 mg L⁻¹. Ve fázi kvetení (od pátého týdne do sklizně) N-NO_3^- 98.87 mg L⁻¹; N-NH_4^+ 5.82 mg L⁻¹; P_2O_5 262.77 mg L⁻¹; K_2O 248.36 mg L⁻¹; CaO 138.31 mg L⁻¹; MgO 85.33 mg L⁻¹; SO_4^{2-} 211.20 mg L⁻¹.

Intenzita světla byla rozdělena do dvou skupin jak pro fázi růstu tak květu, kdy skupina S1 pro fázi růstu byla nastavena na 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, skupina S2 pro fázi růstu 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ a pro fázi květu skupina S1 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ a skupina S2 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Statistická analýza ANOVA nepotvrdila vliv hnojení na výnos květenství nebo obsah kanabinoidů u sledovaných rostlin. Naproti tomu, intenzita světla měla výrazný vliv jak na výnos květenství tak i na obsah sledovaných sekundárních metabolitů, THC, CBD, CBG a CBC, kdy obsah těchto látek vzrostl až o 43 %.

Výsledky potvrzují, že intenzita světla je klíčovým faktorem ovlivňující jak kvantitativní tak i kvalitativní výnos rostlin, zatímco složení rozrozků hnojiv na tyto parametry nemá zdaleka tak vysoký vliv.

Součástí experimentu byla také analýza životního cyklu (LCA) s cílem posoudit environmentální dopady pěstování konopí v řízeném prostředí pro skupiny R1.S1., R1.S2., R2.S1. a R2.S2., kdy bylo potvrzeno, že hlavními faktory ovlivňující environmentální zátěž je spotřeba elektřiny a aplikace hnojiv, přičemž elektřina má na uhlíkovou stopu dominantní vliv. Analýza životního cyklu nicméně paradoxně potvrdila, že vyšší intenzita světla má nižší environmentální dopad než nižší intenzita světla, jelikož přináší větší výnos suchých květenství a tím nižší zátěž na jednotku produkce.

Klíčová slova: Kontrolované prostředí, hnojiva, intenzita světla, LCA

Abstract

The cannabis plant has been cultivated for decades, although the plant is considered an addictive substance in many countries around the world, both as a hemp and medicinal variety. Previously, commonly grown plants were classified as industrial hemp, i.e. cannabis with a THC content of less than 1 %. However, in recent years, it has been possible to commercially grow so-called cannabis for medical use, i.e. cannabis that can contain more than 20 % of this cannabinoid.

In the Czech Republic, cannabis for medical use can only be grown in climate-controlled brick buildings without access to daylight, which means that growers must grow plants differently than in the fields and must control all inputs and parameters, which requires a considerable degree of understanding of the growing system.

One of the most discussed parameters controlled by growers is light, including its quality and quantity, as well as fertilisers. Fertilisers (their different concentrations) and light (its different intensity) were the subject of this thesis.

In this experiment, cannabis plants were grown in a controlled environment, where they were cultivated for twelve weeks overall. These plants were subjected to two regimes of fertilisers, R1 and R2.

Fertiliser regime R1 was applied for the whole growing cycle (vegetation and flowering phase) with parameters N-NO_3^- 131.26 mg L⁻¹; N-NH_4^+ 6.23 mg L⁻¹; P_2O_5 30.85 mg L⁻¹; K_2O 112.46 mg L⁻¹; CaO 147.90 mg L⁻¹; MgO 45.72 mg L⁻¹; SO_4^{2-} 33.79 mg L⁻¹. Fertiliser regime R2 was divided into the vegetation phase and the flowering phase. In the vegetation phase (first four weeks), the fertiliser rate was applied at N-NO_3^- 164.99 mg L⁻¹; N-NH_4^+ 5.28 mg L⁻¹; P_2O_5 65.87 mg L⁻¹; K_2O 228.27 mg L⁻¹; CaO 125.42 mg L⁻¹; MgO 78.93 mg L⁻¹; SO_4^{2-} 50.16 mg L⁻¹. In the flowering phase (from fifth week to harvest), N-NO_3^- 98.87 mg L⁻¹; N-NH_4^+ 5.82 mg L⁻¹; P_2O_5 262.77 mg L⁻¹; K_2O 248.36 mg L⁻¹; CaO 138.31 mg L⁻¹; MgO 85.33 mg L⁻¹; SO_4^{2-} 211.20 mg L⁻¹.

The intensity of the light was divided into two groups for both the growth and flowering phases, where the S1 group for the growth phase was set to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the S2 group for the growth phase to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and for the flowering phase, the S1 group to 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the S2 group to 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The statistical analysis of ANOVA did not confirm the effect of fertilisers on inflorescence yield or cannabinoid content in the monitored plants. On the other hand, the light intensity had a significant effect on both the yield of inflorescences and the

content of the monitored secondary metabolites, THC, CBD, CBG and CBC, with the content of these substances increasing by up to 43 %.

The results confirm that light intensity is a key factor influencing both quantitative and qualitative yield of plants, while the composition of fertiliser spreads does not have such a high effect on these metrics.

The experiment also included a life cycle analysis (LCA) to assess the environmental impacts of growing cannabis in a controlled environment for groups. R1.S1., R1.S2., R2.S1. and R2.S2., where it was confirmed that the main factors affecting the environmental burden are electricity consumption and fertiliser application, with electricity having a dominant impact on the carbon footprint. However, the life cycle analysis paradoxically confirmed that higher light intensity has a lower environmental impact than lower light intensity, as it brings a higher yield of dry inflorescences and thus a lower burden per unit of production.

Keywords: Controlled environment, fertilisers, light intensity, LCA

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Introduction

Cannabis sativa L. has been cultivated for millennia for fibre, seeds and medicinal purposes. Even then, this plant was well known for its psychoactive properties, which is also why this plant was heavily regulated for decades. In recent years, legal frameworks have been gradually changing, allowing for the cultivation of cannabis by private companies, primarily for medicinal purposes, albeit under strict and controlled conditions.

In the Czech Republic, an amendment to the law on psychoactive substances introduced several years ago enabled private companies to cultivate cannabis for medicinal purposes.

Cannabis sativa ssp. sativa or *Cannabis sativa ssp. Indica* or their hybrids have already been cultivated under governmental licenses for at least one decade, mainly in the USA. Licensed companies in Czechia must cultivate cannabis plants in controlled environments without access to daylight, and key parameters, such as temperature and humidity, among others, must be carefully monitored and regulated.

Since *Cannabis* plants for medical purposes are grown under a governmental license, there is a strong emphasis that the processes are reproducible and repeatable. After harvest, the dried inflorescence serves as an intermediate for the Active Pharmaceutical Ingredient (API), which is subsequently used in the manufacture of medicines.

It is therefore expected that harvested inflorescences, before entering the manufacturing process, will have comparable parameters - primarily cannabinoid content, regardless of whether it is the first or twentieth harvest, provided that the same chemotype of *Cannabis sativa* L. is grown. This means that the production conditions, such as watering, temperature, humidity, light intensity, light spectrum, and cultivation media, must remain consistent throughout the cultivation cycle and year.

To date, there is limited scientific information available regarding standardised *Cannabis* cultivation. By contrast, a large body of non-scientific knowledge exists, originating from illegal cultivation during the decades when *Cannabis* cultivation was prohibited. Unfortunately, this knowledge is hardly applicable to the pharmaceutical industry, which requires validated procedures, quality assurance, quality control and scientific evidence rather than empirical practices.

1 Literature Review

The taxonomy of *Cannabis sativa* L. is, even today, not fully harmonised. McPartland (2000) described several species, including *Sativa*, *Indica*, *Ruderalis*, and *Afghanica*. After a few years, Hillig (2005) proposed an alternative classification, suggesting there should be seven taxonomical species, including *Cannabis ruderalis* and *Cannabis sativa* ssp. *sativa*, *Cannabis sativa* ssp. *Spontanea*, *Cannabis indica* ssp. *Karifiristanica*, *Cannabis indica* ssp. *indica*, *Cannabis indica* ssp. *Afghanica* and *Cannabis indica* ssp. *Chinensis*. Although there are several possible classifications, the most accepted and used is *Cannabis sativa* L. ssp. *Sativa*, *Indica* or *Ruderalis* (Hillig, 2005; Small, 2017; McPartland, 2018; Zhang *et al.*, 2018).

Cannabis is a dioecious plant, meaning that the plant bears male and female flowers separately (Fig. 1). This means that to have seed production, male flowers need to pollinate female flowers, which is not desirable in closed cultivation systems, since these systems are used mainly in medical cannabis production (Ilikj *et al.* 2020).



Figure 1: Difference between male and female flowers. Source: Jaroslav Neumann

Cannabis plants are well known for their secondary metabolites, cannabinoids, which are 22-carbon terpene-phenolic compounds unique to *Cannabis sativa* L., and they represent one of the most extensively studied aspects of this plant. The primary psychoactive compound is Δ^9 -tetrahydrocannabinol (THC), widely recognised for its therapeutic potential (Van Bakel *et al.* 2011).

Another recognised compound is cannabidiol (CBD), which is a non-psychoactive cannabinoid that exhibits significant medical properties such as neuroprotection in Alzheimer's disease (Iuvone *et al.* 2004).

Cannabinoids, mainly cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCa) and cannabichromenic acid (CBCa), are synthesised from cannabigerol (CBG), which is synthesised from a geranyl pyrophosphate and olivetolic acid (Flores-Sanchez & Verpoorte, 2008).

Another group of great metabolic importance are isoprenoids, commonly referred to as *terpenes* are prevalent, with over 200 terpenes identified and they are responsible for the flavour and taste (Booth *et al.* 2017).

Since this thesis is focused on the cultivation of medical cannabis, it is required in the Czech Republic to cultivate these plants in buildings without access to daylight.

Given that minimum legal standards must be fulfilled (Decree No. 235 Coll., 2022), that all processes should be repeatable, and considering the nature of the material, a plant-based medical substance primarily intended for inhalation, the need for standardisation of cultivation becomes evident.

Unlike chemically synthesised drugs such as *ibuprofenum*, plant-based medical substances, especially medical cannabis, are not as pure or stable. Their phytochemical composition and overall yield are highly influenced by cultivation conditions such as temperature, humidity, airflow, light, CO₂ concentration, fertilisers and pest control (Chandra *et al.* 2008; Chandra *et al.* 2011; Magagnini *et al.* 2018).

This study aims to investigate how different fertiliser regimes and light intensity affect quantitative parameters (e.g., the yield of dried flowers) and qualitative yield (e.g., the yield of cannabinoids).

For medical substances that have not yet been prescribed as a drug, the homogeneity and repeatability requirements are primarily derived from the European Commission's GMP guidelines (European Commission, 2014).

As mentioned above, in the Czech Republic, medical cannabis can be grown only under a license "*License for the cultivation of cannabis plants for medical use*". After the harvest of this cannabis plant and its packaging, the cannabis inflorescence is considered an intermediate product (Decree No. 235 Coll., 2022).

This intermediate product can then be forwarded to a facility with a GMP (Good Manufacturing Practice) certificate, where it is further processed in accordance

with the European Commission's GMP guidelines. Only GMP-processed cannabis inflorescence can be sent to pharmacies.

From this chapter, one can reasonably assume that the homogeneity must come mainly from the cultivation site, since the cultivation conditions might influence the specification of cannabis flowers the most.

Cannabis cultivation, if being done in a controlled environment, is extremely demanding, mainly in regard to energy inputs (electric energy). The largest contributors to GHG (greenhouse gas) emissions, depending on the region, are HVAC (heating, ventilation, and air conditioning) and lighting.

The second biggest contributor to GHG emissions is light that is required in a controlled environment, as the sole source of light in this particular cultivation system is either LED or HPS fixtures (Wei *et al.* 2021), with LED being the most preferred (Nakai *et al.* 2020; Poulet *et al.* 2014; Zabel *et al.* 2016).

Usually in cannabis cultivation, the intensity of the light is between 50 and 200 times higher than light intensity in the office, and unlike in an office, the light is turned on 12 hours in the flowering phase (Carpentier *et al.* 2012) or 18 to 24 hours in the vegetation phase (Chandra *et al.* 2020).

Controlled cultivation of cannabis can have, for example, 50 fixtures in one production room with the power of 900 watts, which would mean daily consumption around 540 kilowatt-hours in the flowering phase, and during the whole phase (8 weeks), the consumption would be 30,340 kilowatt-hours.

With the legalisation of cannabis, it is possible that energy consumption around the world will noticeably increase, and LCA should be able to help inform authorities in setting proper guidelines for large companies in the cannabis industry to decrease GHG emissions from their production. A great example is Colorado, where the GHG emissions from the cultivation of cannabis in a closed environment are on par with the coal mining industry within that state (Herald, 2019).

2 Aims and Hypotheses

Aims of the thesis

This thesis aims to standardise two factors that influence quantitative (yield of inflorescence) and qualitative (yield of cannabinoids) parameters, specifically nutrition (fertilisers) and light (light intensity). The investigation will examine how nutrient solutions with varying concentrations of nutrients affect the cannabis plant under two different light intensities, as well as how two different light intensities impact the cannabis plant under two different nutrient solutions.

Additionally, the study will investigate whether higher light intensity, when combined with moderate nutrient input, can result in improved input-output efficiency and a lower environmental impact per unit of yield. This reflects the broader aim of evaluating cultivation strategies not only for their agronomic performance, but also for their environmental sustainability.

Hypothesis I.

Fertilisers will have no significant effect on quantitative or qualitative parameters. This will be tested on two different fertiliser regimes, i.e. fertiliser regime I. (R1) and fertiliser regime II. (R2).

Hypothesis II.

Light intensity will have a significant impact on quantitative parameters rather than on qualitative parameters. This will be tested on two different light intensities, i.e. intensity I. (S1) and intensity II. (S2).

Hypothesis III.

Higher light intensity, when combined with the lower fertiliser input, will result in a more environmentally efficient cultivation system, with reduced environmental impact per unit of yield. This will be assessed by comparing scenarios with light input and low fertiliser dose (e.g., R1.S2) against those with high fertiliser input but low intensity lighting (R2.S1), based on key LCA impact categories (GHG emissions, eutrophication, and human toxicity).

3 Materials and Methods

3.1 Experimental design

The practical part of this thesis was carried out at the licensed company ECO GROW s.r.o. in the region of South Bohemia, Czech Republic. There were two overall experiments, and their timeline is shown in Figs. 2 and 3.

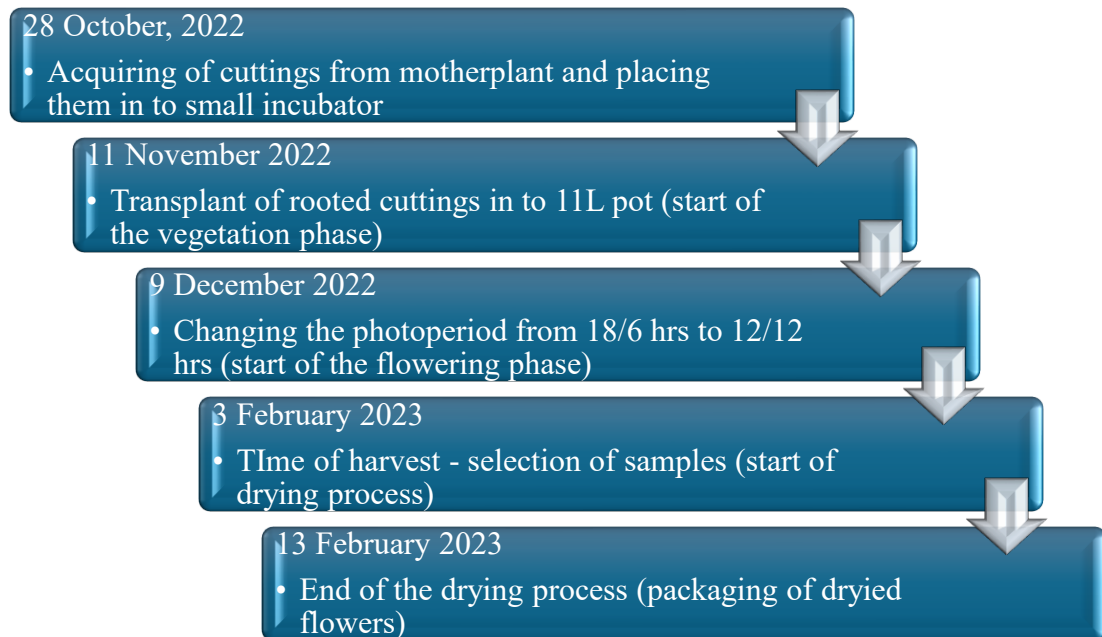


Figure 2: Timeline of the experiment I

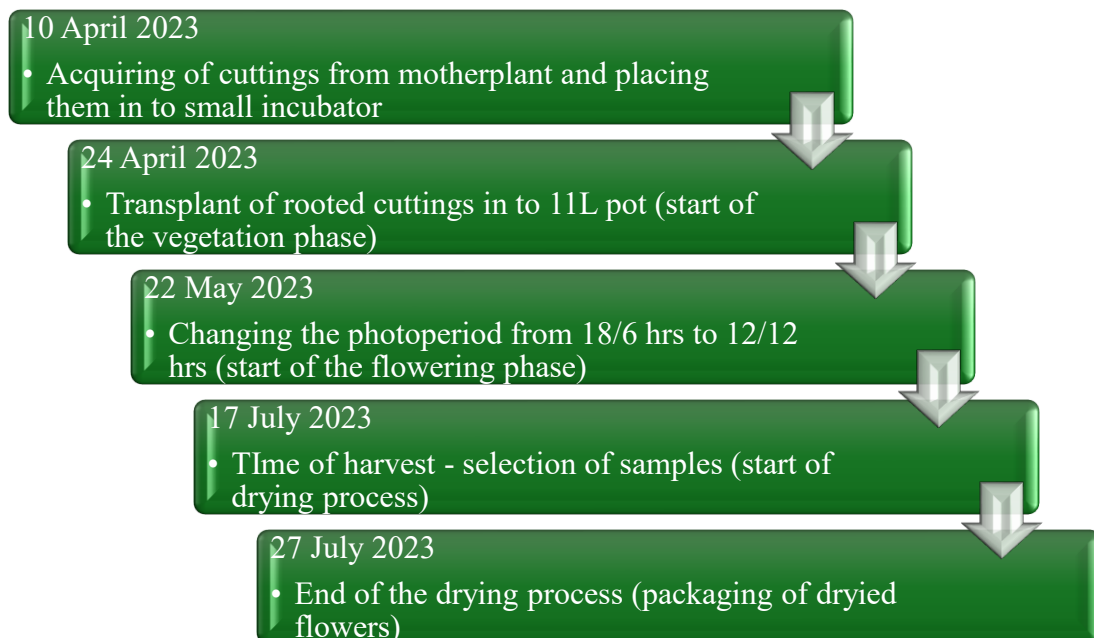


Figure 3: Timeline of the experiment II.

Plants were divided into four groups of 8 plants per group. The group distribution is presented in Fig. 4.

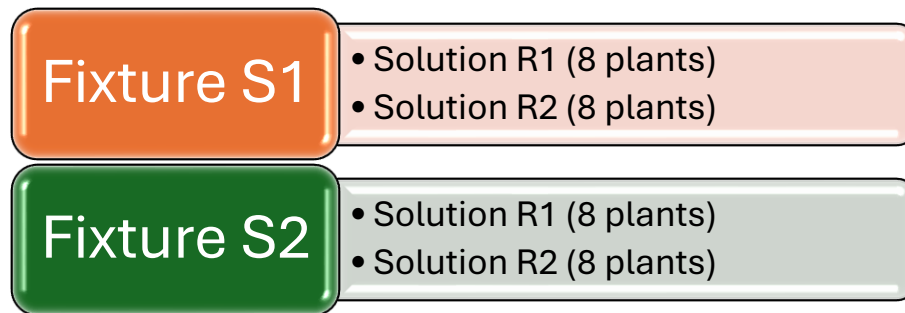


Figure 4: Representation of the division of groups R1.S1., R2.S1., R1.S2, and R2.S2 for experiments 1 and 2.

3.2 Cultivation, harvesting and sample processing

For cultivation purposes of this thesis, one plant was selected as a mother plant. This plant underwent a selection process at the company, so its chemotype and phenotype were well understood. The mother plant used for this experiment is shown in Fig. 5.

The selection of the plant was carried out prior to this thesis. From this mother plant, or more precisely, from cuttings propagated and grown at the company before the start of this experiment, fertiliser regime R1 was formulated based on the water use efficiency and analysis of nutrients in the leaves of previously cultivated plants.

Cuttings were treated with Clonex® Rooting Gel (Growth Technology Ltd., GreatWesternWay, Tanton, UK), a commercially available rooting stimulant containing root hormones, antimicrobial agents, vitamins and nutrients that support root initiation. After the treatment with clones, the cuttings were transferred to Grodan® (Rockwool B.V., Roermond, Netherlands) mineral wool with a size of 40x40x40 mm, which was soaked for 24 hours in pH (5.7) adjusted water.

Cuttings in Grodan® cubes were then put into a greenhouse with dimensions of 540 x 279 x 250 mm. After 14 days, 32 cuttings with a sufficient root system were taken from the greenhouse and transferred into an 11 L plastic pot with 60 % coco peat and 40 % perlite (Gramoflor GmbH & Co. KG, Vechta, Germany) as a medium. With the transplanting of the cuttings, day 1 of the experiment started.



Figure 5: Mother plant used for plant material. Source: Jaroslav Neumann.

The cultivation process consisted of four weeks of the vegetative phase and eight weeks of the flowering phase. The spectrum of the fixture is shown in Fig. 6.

During the vegetation phase, the target PPFD for fixture S1 was set at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ with the power of 360 watts, and for fixture S2, the PPFD was set at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ with the power of 540 watts.

During the flowering phase, the target PPFD for fixture S1 was set at $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ with the power of 540 watts, and for fixture S2, the PPFD was set at $1,300 \mu\text{mol m}^{-2} \text{s}^{-1}$ with the power of 900 watts.

The intensity of the light (PPFD) can be easily adjusted (higher or lower) by changing the distance of the fixture above the plant canopy or increasing the power of the fixture, with a probable adjustment in the height of the fixture farther from the

plant canopy. During the measurements of light intensity, the light fixture was either moved closer or farther from the plant canopy.

Each fixture was measured at five different points using an Apogee MQ610 (Apogee Instruments Inc., Logan, UT, USA) at the bottom left corner, bottom right corner, upper left corner, upper right corner, and in the middle. After calculating the average PPFD using the measurement values from each fixture, they were then rounded to the nearest hundred. Fig. 7 and 8 show measured values for each fixture.

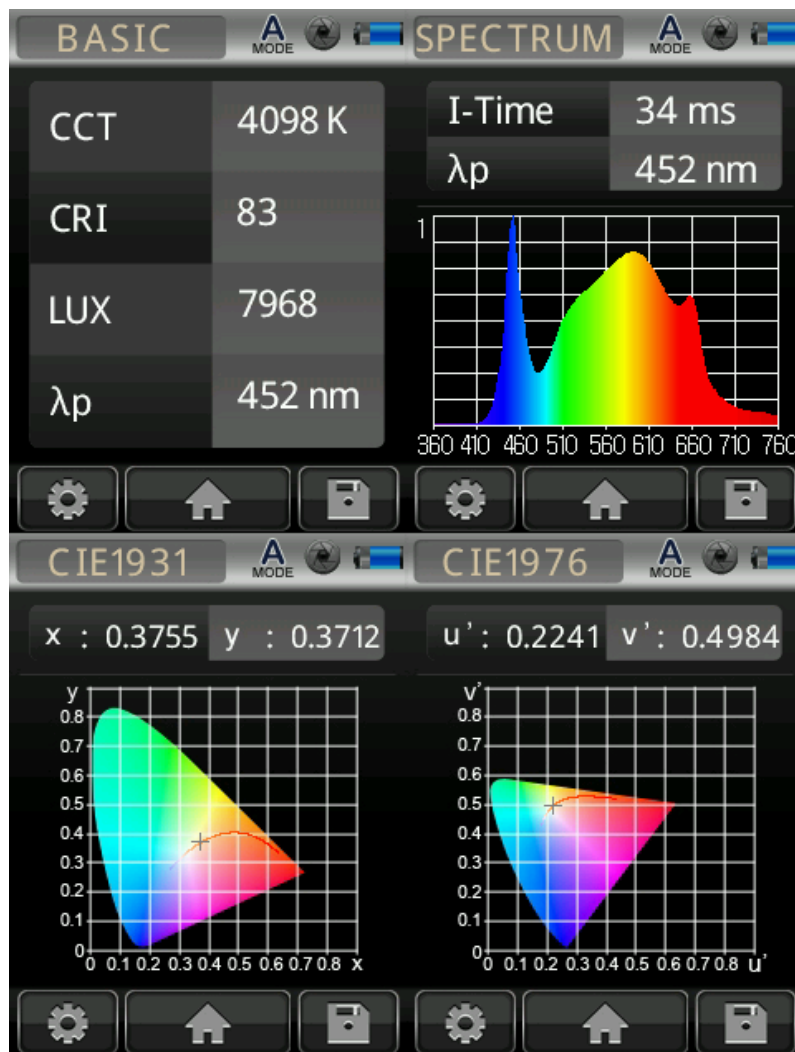


Figure 6: Light spectrum of SunPro Sundocan 900w fixture. Adapted from Konvalina, Neumann *et al.* (2024).

Measured with the UPRtek MK350 LED device (TAIWAN). CTT (correlated colour temperature): 4098 K indicates the colour temperature of the light in Kelvin. Light with a value of 4098 K has a white colour, close to neutral to warm white. CRI (colour rendering index): 83 measures how accurately a given light displays colours compared to natural light. LUX: 7968 indicates the intensity in LUX, where 7958 LUX is considered high intensity. λ_p (Peak

Wavelength): 452 nm means that the dominant wavelength of the light spectrum is 452 nm, which corresponds to the blue part of the spectrum. I-Time: 34 ms is the integration time for the measurement, which was set for 34 ms. CIE1931 (x, y) shows the coordinates $x = 0.3755$ and $y = 0.3712$ that indicate where the point of light is located on the colour diagram, which corresponds to approximately neutral white. CIE1976 (u' , v'): The coordinates $u' = 0.2241$ and $v' = 0.4984$ provide similar information to CIE1931, which better matches human colour perception (taken from Konvalina, Neumann *et al.* 2024).



Figure 7: Experiment 1 (left) and 2 (right), light S1 + nutrients.

Left section: The green background visually separates the fertiliser groups, i.e., the less concentrated fertiliser regime R1 and the concentrated fertiliser regime R2. The blue background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the vegetative phase in Experiment 1. The orange background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the flowering phase in Experiment 1.

Right section: The purple background visually separates the fertiliser groups, i.e., the less concentrated fertiliser regime R1 and the concentrated fertiliser regime R2. The blue background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the vegetative phase in Experiment 2. The orange background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the flowering phase in Experiment 2.



Figure 8: Experiment 1 (left) and 2 (right), light S2 + nutrients.

Left section: The green background visually separates the fertiliser groups, i.e., the less concentrated fertiliser regime R1 and the concentrated fertiliser regime R2. The blue background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the vegetative phase in Experiment 1. The orange background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the flowering phase in Experiment 1.

Right section: The purple background visually separates the fertiliser groups, i.e., the less concentrated fertiliser regime R1 and the concentrated fertiliser regime R2. The blue background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the vegetative phase in Experiment 2. The orange background represents the measured values of light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the flowering phase in Experiment 2.

Only mineral fertilisers were used during experiments. Watering of plants was done in two different ways, namely, during the vegetation phase, plants were watered manually. During the flowering phase, plants were watered via the Autopot® system (AutoPot (Global) Ltd., Hampshire, UK).

The pH value was maintained at 6.1 ± 0.1 throughout experiments 1 and 2. If a fluctuation in pH occurred, a 30 % concentration solution of KOH^- was used for increasing the pH or a 30 % concentration solution of HNO_3^- was used for decreasing the pH.

At the time of harvest, plants were divided based on the treatment combination into four variants: fertiliser 1 + light 1 (R1.S1.), fertiliser 1 + light 2 (R1.S2.), fertiliser 2 + light 1 (R2.S1) and fertiliser 2 + light 2 (R2.S2.). Each treatment group consisted of eight plants. From each group, inflorescences were weighed separately (for each individual plant). After that, samples were randomly taken from each group, primarily the largest and most robust flowers. Harvested inflorescence samples were then dried under controlled conditions (20°C and 50 % relative humidity) for a period of ten days.

After drying, the inflorescence was weighed again, and samples were sent for further processing (analysis) to determine the cannabinoid content. Fig. 9 shows approximately one week before harvest.



Figure 9: Plants before harvest. Source: Jaroslav Neumann.

3.3 Analytical procedures

3.3.1 Quantitative evaluation

After harvest, each plant was trimmed separately to prevent cross-contamination between samples. Dried flowers were weighed individually for each specific group. The weight of dried flowers from individual plants was recorded and used for statistical analysis.

3.3.2 Qualitative evaluation

Samples of dried flowers were sent to the Institut für Hanfanalytik (Vienna, Austria). The equipment used for determining cannabinoid content was an HPLC-DAD (High-Performance Liquid Chromatography - Diode Array Detector), and the method used was in accordance with Ph. Eur., 2.2.29 of the European Pharmacopoeia.

3.4 Life cycle assessment methodology

3.4.1 Goal and scope definition

Part of this thesis involved evaluating the environmental impacts of various cultivation strategies for *Cannabis sativa* L. grown in a controlled environment. The analysis was carried out using the LCA method, in accordance with ISO 14044 standards. Calculations were performed in SimaPro Craft Analyst 10.2.0.0 software; ReCiPe 2016 Midpoint (H) V1.09 / World (2010) H; Cut-off System Model approach; Characterisation model.

The functional unit (FU) was defined as 1 kilogram of dried cannabis inflorescence, representing the primary output of the system. System boundaries included the cultivation phase and immediate post-harvest handling, excluding further processing, packaging, and waste management. Key inputs, such as electricity, water, fertilisers, growing media, and material transport, were taken into account.

Co-products, such as green biomass, were excluded because they were composted without economic allocation. Propagation by cuttings was not assessed, since all treatments were based on genetically identical clones.

This setup enabled a consistent comparison of four cultivation scenarios, differing in light intensity and nutrient input.

3.4.2 Primary and secondary data

Environmental impact assessment in this study was based on empirical data collected from Konvalina, Neumann *et al.* (2024). The experimental design consisted of four treatment groups, which differed in light intensity and nutrient composition. Each treatment was replicated to increase data robustness.

Primary data covered material and energy flows, including water and fertiliser use, substrate components and electricity consumption for lighting, ventilation and drying. The nutrient regimes (R1 and R2) and light regimes (S1 and S2) were designed to reflect different cultivation strategies.

No co-product allocation was applied, and green biomass was assumed to be composted. Data quality was evaluated based on ILCD guidelines, with measurements corresponding to real conditions in the Czech Republic.

To complement the primary inventory, background data were sourced from established LCA databases, including Ecoinvent v3.10, Agri-footprint v6.0, and

AGRIBALYSE. The electricity profile was adjusted to reflect the Czech energy mix of the same year.

3.4.3 Impact assessment categories

The selection of environmental impact categories in this study was inspired by the framework proposed by Dijkman *et al.* (2018), who outlined suitable indicators for evaluating agricultural production systems. Based on their recommendations, the analysis included the following midpoint categories: climate change (expressed in kg CO₂ equivalents), terrestrial acidification (kg SO₂ eq), freshwater eutrophication (kg P eq), marine eutrophication (g N eq), terrestrial and freshwater ecotoxicity (both in g 1,4-DCB eq), water depletion (m³ eq), and human toxicity (kg 1,4-DCB eq). Additionally, land use (m²·year) was included due to its particular significance in crop-based assessments.

To simplify the interpretation of human health-related results, the categories of carcinogenic and non-carcinogenic toxicity were merged into a single indicator of human toxicity. This approach was justified by the fact that both share the same reference substance and have partially overlapping exposure pathways and health implications.

3.5 Statistical analysis

Statistical analyses were conducted to evaluate the impact of light conditions and nutrient treatments on cannabinoid concentrations. All data processing was carried out using JMP version 14 (SAS Institute Inc., Cary, NC, USA). The Tukey's HSD test was applied to determine significant differences between treatment groups, with the significance threshold set at $p < 0.05$. In brief, the experiment was conducted twice, and each plant within the study served as an individual replicate.

The results are presented in two main sections: the first addresses the influence of light regime (S1 and S2) and nutrient solutions (R1 and R2) on plant morphological parameters, the second focuses on the effects of these treatments on flower yield and cannabinoid profiles.

4 Results and Discussion

4.1 Effect of fertilisers and light on yield and cannabinoid content

The statistical analysis (ANOVA) did not confirm any influence of fertiliser solutions on yield or cannabinoid content, as can be seen in Table 1.

Table 1: ANOVA results for the treatment for yield in dry mass (DM) (n=8), Cannabidiol (CBD), Tetrahydrocannabinol (THC), Cannabigerol (CBG) and Cannabichromene (CBC) (n=4) under the effects of nutrition. Adapted from Konvalina, Neumann *et al.* (2024).

Effect		Yield (g DM)	CBD (%)	THC (%)	CBG (%)	CBC (%)
Nutrition	R1	60.85±3.94	10.05±0.65	0.446±0.03	0.846±0.11	0.160±0.02
	R2	58.34±3.57	9.58±0.20	0.428±0.01	0.749±0.08	0.193±0.03
	p-Value	ns	ns	ns	ns	ns

ns is indicated not significant ($p > 0.05$).

Result summarisation of the analysis of variance (ANOVA) for the effect of nutrition treatments on yield (dry mass) and cannabinoid content.

Findings from this study align with those mentioned above and with Massuela *et al.* (2023), who reported that lower fertiliser inputs can maintain CBD yield due to compensatory increases in cannabinoid concentration. The study further confirmed that mineral fertilisers lead to a faster nutrient mobilisation and higher biomass production during late flowering but may also cause a dilution of cannabinoids at higher application rates, as shown by Bernstein *et al.* (2019), who used 17 mg of phosphorus per litter with an additional 2 g of superphosphate. They did not observe any effects compared to control group in regards to flower yield or cannabinoid content, with Cockson *et al.* (2020) who observed a significant increase in plant biomass using up to 23 mg L⁻¹ of P, but flower and cannabinoid yield stopped increasing at 11 mg L⁻¹ of P and finally with Vaezie *et al.* (2021) who did not found any difference in yield of flowers nor cannabinoid yield when they were testing P concentration between 15 to 180 mg L⁻¹.

Furthermore, Saloner and Bernstein (2022b) in their research employed different fertiliser regimes with a particular emphasis on potassium (K). The fertiliser regime was used on different genotypes of cannabis (Royal Medic and Desert Queen). Interestingly, the highest concentration of major acidic cannabinoids (e.g. THCa, CBDa, CBGa, etc.) were detected under the lowest K treatment (15 mg L⁻¹), and

despite visible physiological responses, K supply had only a moderate effect on inflorescence yield. It was proposed that a concentration of K under 60 mg L⁻¹mg L⁻¹ might be too low and could cause stress to the plant, which may explain the highest concentrations of cannabinoids observed under supplementation with 15 mg L⁻¹ K.

Additionally, no beneficial effects were observed when supplementing 60 – 175 mg L⁻¹ K in the studied genotypes.

Regarding the light intensities, the statistical analysis (ANOVA) did confirm a significant difference ($p < 0.0001$) between light intensities S2 and S1, as can be seen in Table 2.

Table 2: ANOVA results for the treatment for yield in dry mass (DM) (n=8), Cannabidiol (CBD), Tetrahydrocannabinol (THC), Cannabigerol (CBG) and Cannabichromene (CBC) (n=4) under the effects of light

Effect	Yield (g DM)	CBD (%)	THC (%)	CBG (%)	CBC (%)
S1	44.30±1.61b	9.04±0.39b	0.400±0.02b	0.670±0.06	0.145±0.02
S2	74.90±3.27a	10.59±0.38a	0.474±0.02a	0.925±0.11	0.208±0.02
Light					
p-Value	**	**	*	†	†

† $p < 0.1$, * $p < 0.05$, and ** $P < 0.01$.

Result summarization of the analysis of variance (ANOVA) for the effect of nutrition treatments on yield (dry mass) and cannabinoid content.

Results shown in Table 2. aligns with the findings of Eaves *et al.* (2020) who tested several light intensities ranging from 490 PPFD to 1024 PPFD in 3 separate runs. They found a strong indication of a strong relationship in all, meaning the vast majority of yield variation can be attributed to light intensity alone. This suggests that, when intensity is held constant, altering the spectral composition of broad-spectrum light has minimal impact on yield

Further, Rodriguez-Morrison *et al.* (2021) in their study examined the relationship between the average PPFD during the 81 day flowering period and three production parameters of *Cannabis sativa* L. “Stillwater”: A. Dry weight of inflorescences, B. harvest index (defined as the ration of total inflorescence dry weight to total aboveground biomass) and C. Density of apical inflorescence based on fresh weight. They found that cannabis yield (dry inflorescence) was increased linearly as PPFD was increased from 120 to 1.800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (see Fig. 28). Cannabinoid yield increased 4.5 times as the PPFD was increased from 120 to 1.800 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Additionally, Llewellyn *et al.* (2022) found in their study that increasing the light intensity 1.6 times leads to increased yield of inflorescence by the same magnitude, which corresponds to findings mentioned above, and also corresponds with findings in this study.

Findings from this study are also in line with findings of other authors (Chandra *et al.* 2008; Saloner and Bernstein, 2020; Vanhove *et al.* 2011; Potter and Duncombe, 2012) who confirmed that higher light intensity equals higher yield of dry inflorescence, however it may contradict with the findings of Vanhove *et al.* (2011) and Potter and Duncombe (2012) who did see a significant effects of the light intensity on the cannabinoid content, but not on their ratios as it is proposed in this study.

Furthermore, Marcelis *et al.* (2006) reported that a 1 % increase in PPFD corresponds to a 1 % increase in inflorescence yield, which supports the findings above and also confirms the results of this study, where a 1 % increase in PPFD led to a 1.55 % increase in dry inflorescence yield.

Taken together, these findings support the growing consensus that light intensity is a key factor in determining yield in medical cannabis cultivation. Results from this study, alongside the findings from Eaves *et al.* (2020), Rodriguez-Morrison *et al.* (2021) and Llewellyn *et al.* (2022), confirm that an increase in PPFD is consistently associated with a proportional increase in dry inflorescence yield. In this study, light treatment S2 led to significantly higher yields and cannabinoid concentrations compared to the S1 group, particularly

It can be confirmed that fertiliser does not have a significant effect on the yield of dry inflorescence and cannabinoid concentrations, as was confirmed by Westmoreland and Bugbee (2022), Saloner and Bernstein (2022a,b), Saloner and Bernstein (2021), Massuela *et al.* (2023), Cockson *et al.* (2020) and Vaezie *et al.* (2021).

Based on the findings from Eaves *et al.* (2019), Rodriguez-Morrison *et al.* (2021), Llewellyn *et al.* (2022), Chandra *et al.* (2008), Saloner and Bernstein (2020), Vanhove *et al.* (2011), Potter and Duncombe (2012), and Marcelis *et al.* (2006), light is the main factor that promotes the yield of dry inflorescence and cannabinoid concentrations, as it is confirmed in this study.

In this study, the main goal was to provide information on how light (its intensity) and nutrient solution would affect the yield of flowers (inflorescence) and the yield of cannabinoids in a controlled environment. This experiment was conducted within a one-year period and was repeated twice.

There are a lot of misconceptions between growers regarding commercial cultivation practices (Westmoreland and Bugbee, 2022), and also the use of WUE, osmotic potential and VPD is not fully integrated. Instead, factors such as light, nutrients, CO₂ concentration, humidity, and temperature are considered the main factors, often without incorporating VPD, and very often without consideration of WUE and the osmotic potential of the fertiliser.

From the year 1950, it was almost impossible to research cannabis due to legal restrictions all around the globe (Small, 2018), and the main source of information was obtained from illegal cultivation (Decorte and Potter, 2015). Some research was done legally; however, instead of medical cannabis, hemp was studied (Caplan *et al.* 2017a).

At present, there is some research being done for medical cannabis cultivation, but it is not as wide as research on field crops, mainly because medical cannabis is grown in controlled environments, which are expensive to set up and require high energy inputs (Mills, 2012).

In addition, the fertiliser industry accounts for approximately 1.2 % of total global energy consumption, and the primary source of phosphorus, a critical macronutrient, is finite (Dijkman *et al.* 2018). While students in agronomy are often taught how to minimise fertiliser use through soil analyses and nutrient planning prior to sowing and while conventional farmers typically apply fertilisers based on this data, the cannabis industry follows a different trajectory. Fertiliser use in the cannabis industry tends to remain constant regardless of actual plant demand, and the sector's contribution to GHG emissions continues to grow. This unoptimized use of fertiliser may accelerate the depletion of limited phosphorus resources without delivering any added value whatsoever. That is why fertilisers used in this thesis were specially designed, and their effects were observed.

4.2 LCA contribution analysis

This section is adapted from the submitted manuscript Kalkušová, Neumann *et al.* (2025).

In various industries, the use of LCA has grown significantly over the past several decades. There is a growing consumer demand for better product information and science-based climate targets (Jensen *et al.* 1998; Morsetto *et al.* 2017; Walenta 2020). According to Sala *et al.* (2021), there is an indicated increase in the use of all life cycle–related approaches in EU policies and communications, which suggests that LCA methodologies and tools in policy support will influence policymaking in the future.

Beyond yield and phytochemical composition, the environmental performance of the different cultivation strategies was assessed using LCA. The analysis was performed per functional unit of 1 kg of dried inflorescence. Fig. 10 and 11 show the contribution analyses, highlighting the relative impact of different inputs and nutrients on selected environmental categories. This allows us to identify which inputs dominate the environmental impact and how fertiliser regimes (R1 vs R2) and light intensity (S1 vs S2) influence the overall sustainability of the system.

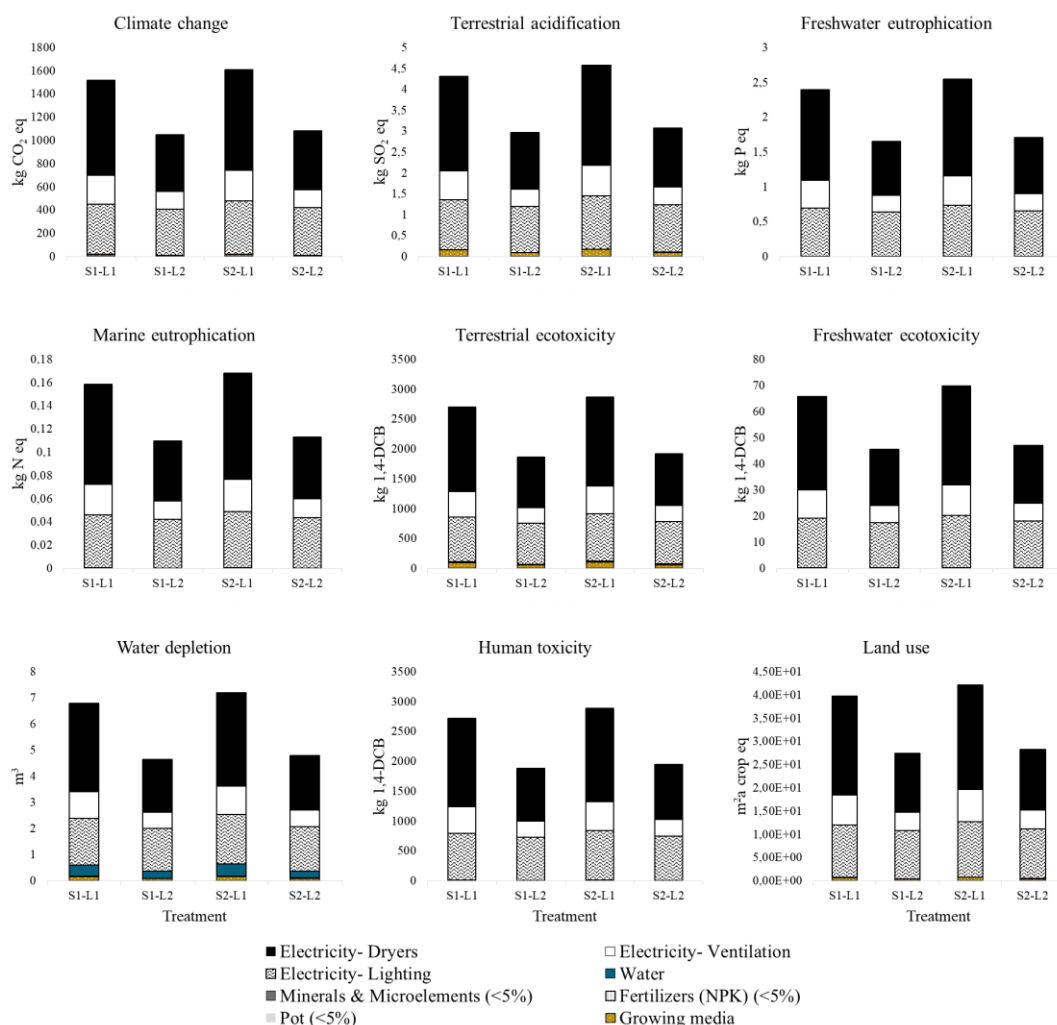


Figure 10: Contribution analyses: Environmental load per 1 kg of dried inflorescence, S1-L1: solution 1, lighting 1, S1-L2: solution 1, lighting 2, S2-L1: solution 2, lighting 1, S2-L2: solution 2, lighting 2, Climate change (kg CO₂ eq), Terrestrial acidification (kg SO₂ eq), Freshwater eutrophication (kg P eq), Marine eutrophication (kg N eq), Terrestrial ecotoxicity (kg 1,4-DCB), Freshwater ecotoxicity (kg 1,4-DCB), Water depletion (m³), Human toxicity (kg 1,4-DCB), Land use (m²a crop eq), “Fertilisers (NPK)” include NO₃⁻, NH₄⁺, P₂O₅, and K₂O fertilisers. “Minerals & Microelements” include calcium carbonate, magnesium oxide, sulphite, and microelements (Fe, B, Cu, Zn, Mn, Mo). “Growing media” include stone wool, coconut fibre, and perlite. Inputs contributing less than 5 % in all categories are marked as ‘<5 %’. SimaPro Craft Analyst 10.2.0.0 software; ReCiPe 2016 Midpoint (H) V1.09 / World (2010) H; Cut-off System Model approach; Characterisation model. Adapted from Kalkušová, Neumann *et al.* (2025).

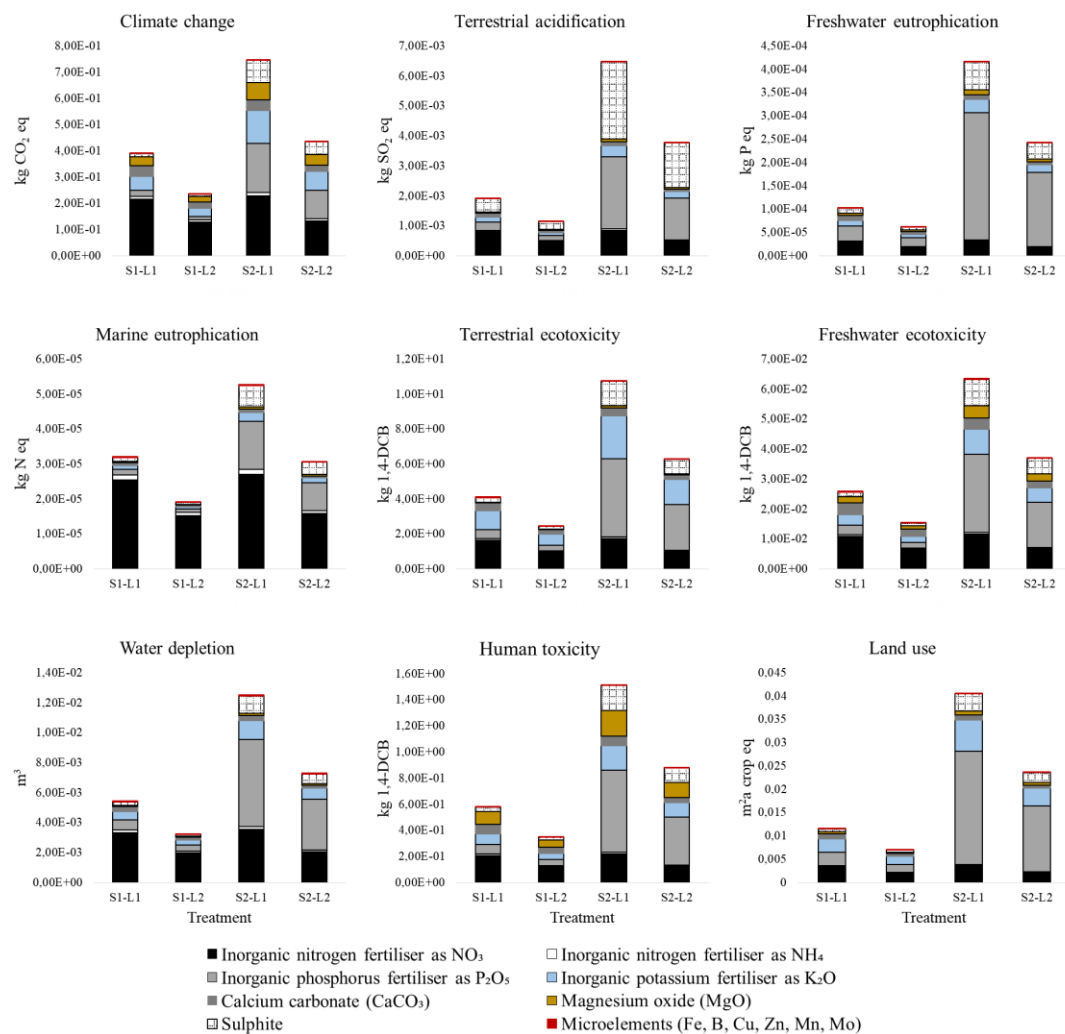


Figure 11: Contribution analyses: Environmental load of nutrients per 1 kg of dried inflorescence, S1-L1: solution 1, lighting 1, S1-L2: solution 1, lighting 2, S2-L1: solution 2, lighting 1, S2-L2: solution 2, lighting 2, Climate change ($\text{kg CO}_2 \text{ eq}$), Terrestrial acidification ($\text{kg SO}_2 \text{ eq}$), Freshwater eutrophication (kg P eq), Marine eutrophication (kg N eq), Terrestrial ecotoxicity (kg 1.4-DCB), Freshwater ecotoxicity (kg 1.4-DCB), Water depletion (m^3), Human toxicity (kg 1.4-DCB), Land use ($\text{m}^2 \text{a crop eq}$), SimaPro Craft Analyst 10.2.0.0 software; ReCiPe 2016 Midpoint (H) V1.09 / World (2010) H; Cut-off System Model approach; Characterization model. Adapted from Kalkušová, Neumann *et al.* (2025).

LCA confirmed that the main drivers of environmental factors in a closed cultivation system are electricity consumption and fertiliser input, which could also be seen in previous chapters, where light (higher light intensity, i.e. higher electricity consumption = higher yield) was proven to be the driving factor of the yield of inflorescence and cannabinoids. Compared to the literature, Summers *et al.* (2021) and Mills (2012) report that CO_2 emissions generally range from 2000 to 5000 $\text{kg CO}_2 \text{ eq}$ per kg of dried cannabis inflorescence, which in this study was significantly lower, up

to 1610 kg CO₂ eq per kg of dried cannabis inflorescence. However, the difference in CO₂ eq per kg was significantly lower, the trend was the same, and electricity has the biggest impact on the carbon footprint. One of the reasons why electricity is the biggest contributor is the fact that the Czech energy mix contributes a lot more than renewable energy sources, since in Czechia, lignite-based electricity is the main source (Šerešová *et al.* 2020). Furthermore, the results reveal a paradoxical outcome, i.e., increased energy consumption has a lower environmental impact (based on the yield of inflorescence) than lower energy consumption; therefore, the CO₂ equivalent per kilogram of biomass is reduced. This supports Hypothesis III and confirms the results from the ANOVA analysis in the sections above. Additionally, the proposal of Poore & Nemecek (2018) states that nitrogen and phosphorus are the major contributors to water eutrophication in the agricultural industry.

5 Conclusion

5.1 Summary of key results

This thesis examined the impact of light intensity and fertiliser composition on the yield and phytochemical profile of *Cannabis sativa* L. grown under fully controlled conditions in a closed environment, while assessing the environmental performance of the cultivation scenarios through LCA.

Light intensity was identified as a key factor for yield, with significantly higher biomass obtained under higher intensity (S2), regardless of fertiliser regime. This effect was statistically significant ($p < 0.00001$).

Fertiliser (R1 vs R2) showed no statistically significant influence on either yield or cannabinoid content (CBD, THC, CBG, CBC), suggesting that lower fertiliser input does not compromise crop performance.

LCA revealed that the most environmentally efficient scenario was R1.S2 (low concentration of fertiliser and high light intensity), due to lower resource inputs per unit of yield. In contrast, high fertiliser treatments (R2) had disproportionally higher environmental impact without notable yield benefits.

5.2 Implications and suggestions for future research

The findings of this thesis have several implications for both the cannabis industry and controlled environmental agriculture:

- a. Growers may reduce fertiliser input without compromising the yield of inflorescence and cannabinoids, helping to reduce environmental impact and cost of the fertilisers.
- b. The interaction of WUE, VPD and osmotic potential could help growers to optimise their production.

Future research should aim to:

- a. Create a fertiliser formula based on WUE for individual cannabis strains and explore their effects not only on the yield of inflorescence and cannabinoids but also on terpenes.
- b. Cooperation with private companies to better understand the needs regarding commercial cultivation of cannabis, with emphasis on reducing inputs in the cultivation and therefore on the environment.

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7 Appendices

Appendix I. List of publications (2020 - 2025)

1. **Neumann. J.**, Líbalová Š.K., Konvalina P., Smetana P., Vráblík P., Šoch M. (2024). *Tribulus terrestris* L. – A review / Kotvičník zemní – přehled. Journal of Central European Agriculture, 25(1), 194-208. <https://doi.org/10.5513/JCEA01/25.1.4119>
2. Konvalina, P., **Neumann, J.**, Hoang, T. N., Bernas, J., Trojan, V., Kuchař, M., Lošát, T., Varga, L. (2024). Effect of Light Intensity and Two Different Nutrient Solutions on the Yield of Flowers and Cannabinoids in Cannabis sativa L. Grown in Controlled Environment. *Agronomy*, 14(12), 2960. <https://doi.org/10.3390/agronomy14122960>
3. **Submitted** - Kalkušová A., **Neumann J.**, Veselovská N., Kůrková E., Konvalina P., Neugschwandtner R., Bernas J. (2025). Rethinking Efficiency: How Increased Electricity Use Can Reduce Environmental Impacts in Controlled Hemp Cultivation.

Conference presentations (proceedings abstracts, paper)

1. **Neumann. J.**, Kužel. S. (2020). The influence of acetylsalicylic acid on the content of diosgenin in *Tribulus terrestris* L. plant. In *Proceedings of the 44th Conference for Students of Agriculture and Veterinary Medicine with International Participation*, 183-188.
2. **Neumann J.**, Konvalina P. (2023). Elicitation of hemp under LED and its effect on cannabinoid content. In *Proceedings of the 47th Conference for Students of Agriculture and Veterinary Medicine with International Participation*, 73-82.

Books and book chapters

1. Bernas, J., Konvalina, P., **Neumann, J.** (2024). Climate resilient cropping systems. In E. Bilen (Ed.), *Climate focused agricultural vocational education (CLIAVE)* (pp. 2–57). Atatürk Horticultural Central Research Institute. ISBN 978-625-94899-5-7.

Appendix II. Training and supervision plan during the study

Name	Jaroslav Neumann
Department	Agroecosystems
Supervisor	doc. Ing. Petr Konvalina, Ph.D.
Period	2020 - present
PhD courses	(Year/Semester)
Plant nutrition and fertilization (<i>Výživa rostlin a hnojení</i>)	2021 / SS
Plant physiology (<i>Fyziologie rostlin</i>)	2024 / SS
Current trends in crop production (<i>Aktuální trendy v rostlinné výrobě</i>)	2024 / SS
Chemistry of agricultural products (<i>Chemie zemědělských produktů</i>)	2025 / SS
English Language (<i>Anglický jazyk</i>)	2022 / SS
Scientific seminars	
44 th Conference for Students of Agriculture and Veterinary Medicine with International Participation (online), University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Srbija	2020 / WS
Over the Horizon and for Mutual Acquaintance 2 nd USB Conference of Doctoral Students, České Budějovice, Czech Republic (7 – 8 th December 2022).	2022 / WS
47 th Conference for Students of Agriculture and Veterinary Medicine with International Participation, University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Srbija	2023 / WS
Over the Horizon and for Mutual Acquaintance 3 rd USB Conference of Doctoral Students, České Budějovice, Czech Republic (21 – 22 nd November 2023).	2023 / WS
Department seminar, České Budějovice, Czech Republic	2023 / WS
Traning	
Eco Trading spol. s r.o., Štěpánovice 234, 373 73 Štěpánovice, České Budějovice	1 st June 2020 – 19 th June 2020
German Medical GmbH, Friedrich-Ebert-Anlage 36, 60325 Frankfurt am Main	3 rd January 2024 - 1 st February 2024
Lectures	
Organic Plant Production	2022 – 2025
Quality, Processing and Marketing of Bioproduction	2022 – 2025

Appendix III. *Curriculum vitae*

Personal information

Name	Jaroslav
Surname	Neumann
Title	Ing.
Born	20 th August 1994, Klatovy, Czechia
Nationality	Czech
English	English (C1 level), Czech
Contact	neumaj01@fzt.jcu.cz



Education

2020 – present	Ph.D. student of Plant Science at the Faculty of Agriculture and Technology, University of South Bohemia in Ceske Budejovice
2018 – 2020	Ing. from Agroecology at the Faculty of Agriculture, University of South Bohemia in Ceske Budejovice, Czechia
2014 – 2018	Bc. from Sustainable Systems in Agricultural Land at the Faculty of Agriculture, University of South Bohemia in Ceske Budejovice, Czechia

Project participation

2022 - 2024	Erasmus+ KA220-VET - Cooperation partnerships in vocational education and training; Project Title: Climate Focused Agricultural Vocational Education (CLIAVE) – Technician, Teacher/Trainer, Management
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